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Review

Functional Oligosaccharide and Its New Aspect as Immune Modulation

Kodo Otaka

Dept. Cellular Macromolecule Chemistry, Graduate School of Agriculture, Kyoto Prefectural University, 1-5, Hangi-cho, Shimogamo, Sakyo-ku, Kyoto 606-8522, Japan.

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Short chain saccharides have beneficial functions for health of humans and animals, and are known as functional oligosaccharides. They have been utilized as a food ingredient. Recently, new functions of oligosaccharides have been reported from the field of immune study. In this article, I would like to review the immune enhancing ability of β-glucan oligosaccharides extended from laminarin and yeast cell wall based upon recent reports including our own results. It has been shown that β-glucan oligosaccharides possess an activity to stimulate a secretion of cytokine such as TNF-α from human monocytes or macrophage. In addition, oligosaccharides extending from yeast cell wall, prepared by the method of autolysis, together with digestion by endo-1,3-β-glucanase, have enhanced the modulation of immune system when orally administered as a diet ingredient. It is further discussed about the most effective chain length or structure of β-glucan oligosaccharides for the stimulation of monocytes. The yeast cell wall hydrolyzate that contains oligosaccharide can be supplied with easy process. Such hydrolyzate might become promising for the product of practical use in humans and animals.

Keywords: oligosaccharide, β-glucan, laminarin, yeast cell wall, immune modulation

Introduction

Oligosaccharides, a short chain saccharide containing of homo- or hetero-sugars, are well known for their beneficial effects on human life and have been extensively utilized for long time [1]. Functional oligosaccharides, which have a physiological function such as low cariogenicity [2] and bifidobacteria growth factor [3], improve the health of humans and animals. The development of oligosaccharide production requires the identification of new enzymes and application technology of these enzymes because these oligosaccharides are produced from respective polysaccharides or sugars by enzymatic hydrolysis and/or transfer reaction. For instance, maltooligosylsucrose is produced by the transglucosylation reaction of cyclodextrin glucanotransferase from starch and sucrose, and galactooligosaccharides are produced by the hydrolysis and transgalactosylation reaction of lactase from lactose. The prevention of an infection from pathogenic microbes and viruses is important to maintain human health. β-Glucan, which is derived from the yeast cell wall and fungi, is known to possess antimicrobial and antitumor activities through the enhancement of the host immune function. Recently, new functions of oligosaccharides, which have the ability to modulate the immune system in humans, animals, and fish, have been reported. For instance, it has been suggested that mannanoligosaccharide (MO) tends to modulate the systemic immunity, and it has been demonstrated that laminarin oligosaccharide, which is an extension from laminarin hydrolyzed with endo-1,3-β-glucanase, possesses the ability to induce production of cytokines such as TNF-α from human monocytes. Identifying the new functions of oligosaccharides is important; furthermore, it would be of interest to determine whether oligosaccharides or solubilized saccharides from the yeast cell wall can stimulate

Corresponding to: E-mail:
ohtakakpu@yahoo.co.jp

the immune system when they are applied as a supplement like diet ingredient. In this review, the physiological functions of oligosaccharides are reviewed, including enzymes involved in the oligosaccharide production process, and especially the immune modulation ability of oligosaccharides such as MO, laminarin oligosaccharides, and oligosaccharides that are an extension from the yeast cell wall. The elucidation of the characteristics and immune modulation ability of the oligosaccharides from laminarin and water-soluble saccharides and oligosaccharides from yeast, *Hansenula anomala*, is the main focus of this review.

Low-cariogenic oligosaccharides

The prevention of dental caries is a serious problem in our daily life. Oral microbials have been shown to cause dental caries. The bacterium *Streptococci*, including *Streptococcus mutans*, produces a water-insoluble, highly sticky dextran-like glucan from sucrose. The reaction is catalyzed by glucosyltransferase. The glucan covers the dental surface, forming a caries-conductive plaque. The presence of a saccharide such as glucose, fructose or sucrose during plaque formation leads to bacterial acid production in the plaque, subsequently initiating the occurrence of a dental caries [4,5]. Dextranase, which hydrolyzes polysaccharides produced by oral *Streptococii*, may be useful in the prevention of dental plaque [6], and has been used extensively as an ingredient of toothpaste. Similarly, oligosaccharides, including maltooligosylsucrose known as Coupling sugar, prevent dental caries. Those are termed cariogenic oligosaccharides, and the effect of Coupling sugar has been extensively studied. The result indicates that Coupling sugar is not only unavailable for the synthesis of insoluble glucan but also inhibits the synthesis of insoluble glucan from sucrose by *St. mutans*. Furthermore, its acid production is low compared to that from sucrose [2]. Coupling sugar is produced by a transfer reaction catalyzed by the enzyme, cyclomaltodextrin glucanotransferase, which has the ability to convert starch to cyclodextrin and also catalyzes the transglucosylation reaction whereby glycosyl moieties are transferred from starch to an accepter. When the enzyme is incubated with the mixture of starch and sucrose, various maltooligosylsucroses are produced [7].

Palatinose, 6-O-α-D-Glucosyl-D-fructofranose (isomalturose), which has a function as a non-cariogenic sweetener through the suppression of the production of water-insoluble glucans, is mainly utilized in confectionery as a sugar substitute [8-10]. α-Glucosyltransferase derived from bacteria catalyzes the transformation of sucrose to palatinose. When a sucrose solution is passed through the immobilized cell of *Erwinia rapontici*, which exhibits an α-glucosyltransferase activity, sucrose is transformed to palatinose and related oligosaccharides [11].

Bifidobacteria growth factor

Gibson and Roberfroid introduced the concept of "prebiotics" which alter the microbial populations in the intestines and, consequently, improve the health of the host [12]. By definition, prebiotics mean nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one bacterium or a limited number of bacteria in the colon; thus improving host health. Fractooligosaccharides (FO) are nondigestable, but they are selectively utilized by bifidobacteria in human intestinal canal [13,14]. β-Fructofranosidase, derived from *Aspergillus niger*, reacts with sucrose and produces some FO such as 1-kestose (GF2), nystose (GF3) and 1F-β-fructofranosylnystose (GF4) [15]. The administration of FO has been reported to be effective in suppressing the production of intestinal putrefactive substances in both humans and animals. In the case of hyperlipemia patients, the administration of FO greatly improved the level of lipids in serum such as cholesterol and triglyceride as well as the blood pressure [16].

Galactooligosaccharides have a function as bifidobacteria growth factor [17]. β-Galactosidase (lactase), derived from *Aspergillus oryzae*, catalyzes the transgalactosylation reaction as well as the hydrolysis of lactose to produce disaccharide (galactosyl glucose and galactosyl galactose), trisaccharide, and higher oligosaccharide [18]. 6'-Galactosyllactose occurs naturally in human milk and has been utilized as milk replacement inclusion for infants. Further

development of the enzymes and bioengineering to produce these beneficial oligosaccharides in an industrial scale are expected to contribute to the maintenance of human health.

Modulation of the immune system with saccharides and oligosaccharides

Mannanoligosaccharides (MOs)

Supplementation of mannans, which have been reported to modulate the immune system, increases the IgA concentration in the cecal contents of rats [19], bile IgA and systemic IgG in turkeys [20], and neutrophile activity in dogs [21] and fish [22]. Secretory IgA is important in mucosal immunity because it inhibits the attachment and penetration of bacteria in the rumen, increases mucus secretion and prevents inflammatory reactions that would cause damage to the epithelial tissues [23]. On the other hand, the roll of MOs in the modulation of the immune system is not completely understood. It has been reported that MOs tend to enhance microbial populations and modulate the systemic immunity. Dogs supplemented with MOs had lower fecal total aerobes and tended to have a greater *Lactobacillus* population. Furthermore, they were likely to have an enhanced immune system with increased IgA and lymphocyte concentration. The trends of increased serum IgA and lymphocyte concentration are likely due to the increased proliferation of B lymphocytes and secretory IgA occurring in the intestine [24].

β-Glucan oligosaccharides

β-Glucans are mainly distributed among fungi, brown seaweed, lichen and barley as cell wall components of fungi or plant storage substances. Numerous reports have indicated that β-glucans have an activity to stimulate immunity and decrease infectious complications in humans [25,26] and animals [27], and are also known to activate macrophages, neutrophiles, and NK cells by binding to the β-glucan receptor on the cells [28,29]. Immunologically active glucans have non-branched β-1,3-linked backbone or β-1,3-linked backbone with β-1,6-branches. Furthermore, the immunological activities of β-glucans are considered to depend on the structure of glucans, including the molecular mass, degree of branching, length of branch, and higher order structure [30-32]. The first step in macrophage activation by β-1,3-glucan is thought to be the binding of the polymer to specific macrophage receptors and is followed by the secretion of cytokines such as TNF-α. A relatively wide range of β-glucans seems to be recognized by specific receptors, such as CR3 (CD11b/CD18) and dectin-1 [33-35]. However, most of the β-glucans analyzed so far have a relative higher molecular mass, and only limited information is available on the β-glucan with low molecular mass in terms of the correlation between its immunomodulatory function and structure.

Engstad and Robertson reported that the Atlantic salmon macrophage possesses a receptor that recognizes even very short β-1,3-linked glycosyl-chains originated from yeast cell walls [36]. The Atlantic salmon receptor recognizes small oligomers from formolyzed β-glucan particles and linear β-1,3-linked oligomers with a degree of polymerization (DP) ≥ 3. The recognized ability of laminarin and laminaripentaose is abolished by degrading the nonreducing terminal end by means of a sodium periodate treatment. In contrast, in the case of laminarin, this ability is regained by a complete Smith degradation. In addition, it has been shown that salmon macrophage does not recognize peroxidate-oxidized glucan, however, this ability is regained when the glucans are hydrolyzed to recover the nonreducing terminal end. These findings will be very important in future studies. Recently, the β-1,3-glucan oligomer (DP ≥ 4) from laminarin derived from *Laminaria digitata* hydrolyzed with endo-1,3-β-glucanase from *Bacillus clausii* NM-1 is examined with regard to its effect on human peripheral blood monocytes. A conditioned medium prepared by incubating monocytes with the β-1,3-glucan oligomer showed strong inhibitory activity against the proliferation of human leukemia U937 cells. On the other hand, a conditioned medium prepared with original laminarin has little effect on the growth of U937 cells. The cytotoxicity of the monocyte-conditioned medium prepared with the β-1,3-glucan oligomer was significantly reduced by an anti-TNF-α antibody, suggesting that the enzymatically depolymelized β-1,3-glucan oligomer induces TNF-α production in human

monocytes. These results suggest that the β-1,3-glucan oligomer prepared with 1,3-β-glucanase contains an essential structural unit to stimulate macrophage to release TNF-α [37].

We examined the human monocyte-stimulating ability of laminarin from *Eisenia bycyclis* (LB), lichenan from *Cetraria islandica*, and their oligomers depolymerized with purified endo-1,3-β-glucanase from *Arthrobacter* sp. [38]. The monocyte-conditioned medium pre-cultured with a fraction of β-glucan oligomer (DP ≥ 8) from laminarin, LB-1, showed inhibitory activity against the proliferation of human myeloid leukemia U937 cells (Fig. 1). On the other hand, the monocyte-conditioned medium pre-cultured with other β-glucan oligomers and original laminarin and lichenan showed little or no activity. These results suggested that enzymatic hydrolysis is an essential step for the monocyte-stimulation activity of laminarin. Moreover, the average DP of LB-1 (DP ≥ 8) was estimated to be 13, and the ratio of β-1,3- to β-1,6-linkage was estimated to be 1.3 by NMR analysis. Since the average DP and the ratio of β-1,3- to β-1,6-linkage of original laminarin are estimated to be 36 and 1.5 respectively, the structure of the active β-glucan oligomer will have a higher ratio of β-1,6-linkage than that of the original laminarin. These results indicate that the

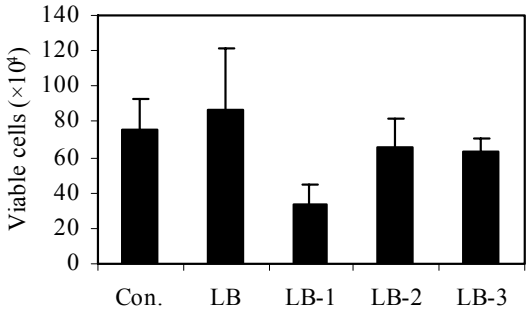

Figure 1. Effects of the monocyte-conditioned medium pre-cultured in the presence or absence of β-glucans on the proliferation of U937 cells. LB-1, -2, -3 are depolymerized oligomers of LB, and their DP values are ≥ 8, 6-7, and 4-5, respectively.

β-glucan oligomer with higher contents of β-1,6-linkage stimulates monocytes to inhibit the proliferation of U937 cells [35]. It is interesting to note that a recently identified β-glucan receptor, dectin-1, can recognize not only β-1,3 but also β-1,6-linked glucans. Although the correlation of β-glucan oligomer with dectin-1 is unknown, the binding of β-glucan to one of the β-glucan receptors may induce cytokine release. It is not clear why the hydrolysis of laminarin is essential for the monocyte-stimulation activity; however, a significant correlation can be expected from the case of Atlantic salmon macrophage mentioned above.

Preparation of soluble saccharide from yeast cell wall

The cell wall of the yeast *Saccharomyces cerevisiae* contains β-glucan, chitin, and mannoprotein, and the β-glucan consists of alkali-insoluble and alkali-soluble components. Yeast β-glucan has been rendered soluble by sulfornylation [39], sulfation [40], phosphorylation [41], and alkali extraction methods for clinical use [42]. We have developed the autolysis and enzymatic hydrolysis method to obtain soluble saccharides from newly isolated yeast *Hansenula anomala* SB1020. Autophagic death occurs when vacuolar hydrolytic protease causes the dissolution of the protoplasm, and autolysis occurs when carbohydrases cause the lysis of the envelope. Glucanase, chitinase, and mannanase participate in yeast autolysis as the lytic enzymes of cell wall [43]. In the progress of autolysis with the heat treatment, a decrease in the alkali-insoluble fraction and an increase in the alkali-soluble fraction were observed, but a small amount of soluble saccharides was released. It is interesting that autolysis in a mixture of two strains, *H. anomala* and *R. glutisis*, was stimulated with synergistic effect, suggesting that *R. glutisis* has strongly reactive carbohydrases and stimulates the hydrolysis of the yeast cell wall. An autolysis product of yeast, *H. anomala* (YHA), stimulates the enzyme reaction of *Rhizopus* sp., which contains endo-1,3-glucanase and protease, but little stimulation was observed without autolysis. These results indicated that enzymatic hydrolysis should be performed after the autolysis, as a significant amount of soluble saccharides can thus be obtained. It was confirmed by NMR and HPLC analyses that the saccharides, enzymatic hydrolysis product of YHA (YHAE), are composed of mono-, oligo-, and polysaccharides, including β-1,3- and

β-1,6-glucosidic bonds, and α-1,2 and α-1,6 linkage [44].

Functions of soluble saccharides from the yeast cell wall

The supplementation of feed including YHA markedly improved the feed efficiency of the juvenile aqua culture ayu, *Plecoglossus altivelis* [45]. It was deduced that the increase in the feed efficiency resulted not from the nutritional value of the ingredients such as vitamins and amino acids but from an addition of a growth-promoting factor or as a result of the enhancement of immunity like protective function against infection of pathogenic microbes. In this regard, the elevation of immunity by the supply of feed or diet including *H. anomala* without autolysis (HA) or YHAE in fish and rats has been reported. The survival rate of the red sea bream, *Pagrus major*, against virulent bacteria, *Vibrio angeulum*, injected intraperitoneally, improved by supplying a feed containing HA in advance. Furthermore, the enhancement of peritoneal macrophages of ddY female rats fed a diet including YHAE was detected in the ability of

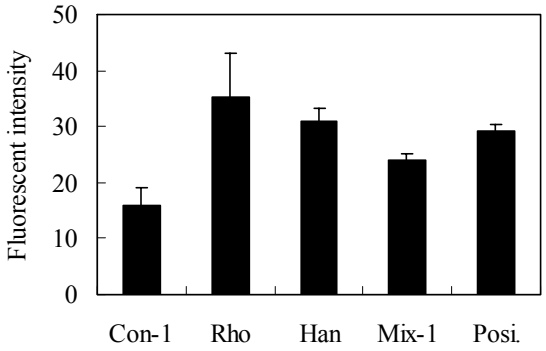

Figure 2. Effects of yeast supplementation on phagocytosis activity of peritoneal macrophage (ddY female rats). Con.; control, Rho; supplementation of *Rhodotorula glutinis*, Han; supplementation of *Hansenula anomala*, Mix-1; supplementation of mixture of *Rhodotorula glutinis* and *Hansenula anomala*, Posi.; treatment with LPS. Values are means ± SD, n = 3.

phagocytosis as well as cathepsin activity and NO production (Fig. 2) [46]. The absorption of these saccharides through the intestines is critical for the elevation of immunity, but the results suggest that oral administration of a yeast autolysis product would modulate the immune system of animals and fish. In addition, YHAE was subjected to immune stimulation examination *in vitro*. YHAE was fractionated through a Sephadex G15 column and each fraction of oligosaccharide and higher molecular mass was submitted to testing. The monocyte-conditioned medium pre-cultured with a fraction of oligosaccharides (Main DP = 5~6), showed a strong inhibitory activity against the proliferation of human myeloid leukemia U937 cells (unpublished results).

All of these results suggest that YHA and YHAE, which include mannan, β-glucan, and its oligomer, are promising products to elevate the immune efficiency and are expected to maintain intestinal health as “prebiotics” for humans and animals. Because YHAE can be easily prepared, it should be useful for commercial applications such as health food products.

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